Rec'd PCT/PTO 2 0 DEC 2004   10/519167

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BINDING A TARGET SUBSTANCE

Field of the Invention

The present invention relates to magnetic particles capable of binding a target substance such as nucleic acid, a process for making such magnetic particles, and a process for isolating a target substance from a target substance-containing sample.

Background to the Invention

Procedures involving nucleic acids such as DNA and RNA continue to play a crucial role in biotechnology. Nucleic acid detection and manipulation including hybridisation, amplification, sequencing and other processes generally require nucleic acid to have been isolated from contaminating material. Where a nucleic acid-containing sample is a biological sample, contaminating material may include proteins, carbohydrates, lipids and polyphenols. Accordingly, a variety of approaches have hitherto been used in the isolation of DNA or RNA.

Early methods of isolating nucleic acid involved a series of extractions with organic solvents, involving ethanol precipitation and dialysis of the nucleic acids. These early methods are relatively laborious and time-consuming and may result in low yield. Isopropanol may also be used in the precipitation of the nucleic acid.

An alcohol precipitation method is described in US5523231. Nucleic acid is precipitated by highly concentrated alcohol in the presence of magnetic beads.

·The precipitate can be separated from supernatant by the application of a magnetic field.

US5395498 describes a method ·for isolating biological macromolecules from electrophoretograms using a matrix of magnetic particles which have an affinity to the molecules separated on the electrophoretogram. Magnetic particles are described with a range of various magnetic substances, those having essentially no magnetic memory, being preferred. A magnetic field is used to attract the magnetic particles to a specific location in the electrophoretogram for specific binding of the particles to a specific species of biological macromolecule, typically separated as a band in the electrophoretogram.

US6027945 describes a method which uses a silica-based nucleic acid binding solid phase in the presence of a chaotrope to isolate nucleic acid. According to this method, the silica-based solid phase is magnetic, thereby facilitating separation of the solid phase containing the target nucleic acid from the liquid phase containing contaminants upon application of a magnetic field. A similar method is described in US5945525.

US 5990302 describes a method for isolating RNA which is also performed in the presence of a chaotrope. A sample is mixed with an acidic solution containing a lithium salt, a chaotropic agent and a nucleic acid-binding carrier to absorb the RNA onto the carrier. The RNA-bound carrier is isolated from the liquid phase and eluted. Magnetic silica particles are used as the nucleic acid-binding carrier, although silica, cellulose,

nitrocellulose, latex and hydroxyapatite are all
mentioned as possible carriers.

WO96/18731 also uses magnetic particles to bind nucleic
acid. In this disclosure the magnetic particles are
polystyrene-based and polyurethane-coated and a detergent
is used instead of a chaotrope.

US 5705628 discloses a method of separating
polynucleotides, especially DNA, by binding the
polynucleotides to a magnetic micro particle having a
functional group-coated surface.

All of the prior art documents described herein and each
of their commercial counterparts known to the present
applicants use magnetic particles which are capable of
being magnetised in the presence of a magnetic field but
which are not themselves magnetic in the absence of such
a field. Paramagnetic or superparamagnetic materials
possess these qualities. It has hitherto been thought
that particles which are themselves magnetic in the
absence of a magnetic field (and which are known as
remanent particles) are undesirable because they
disadvantageously form aggregates because of their
remanence. These aggregates prevent intimate mixture
with sample and are therefore considered to inhibit
partially binding of nucleic acid from the sample to the
magnetic particles.

Contrary to this generally-held thinking, the present
applicants have surprisingly found that remanent magnetic

particles may be advantageously used in isolating nucleic acid and other target substances.

Summary of the Invention

Accordingly, in a first aspect, the present invention provides magnetic particles capable of binding a target substance, which comprise a magnetic material and a matrix material, wherein the magnetic material is remanent upon exposure to a magnetic field and the matrix material has a surface comprising functional groups which promote disaggregation of the particles in the presence of a liquid phase.

It has surprisingly been found that remanent magnetic particles can be extremely effective in separation or isolation of target substances from a sample. Remanent magnetic particles according to the present invention may form aggregates when suspended in a liquid phase but are readily dispersible upon application of a force to disrupt the aggregates. Advantageously, the matrix material of the magnetic particles has a surface comprising functional groups which promote this aggregation of the particles in the presence of the liquid phase.

Because the magnetic particles are remanent, they are highly responsive to magnetic fields. The particles can be made smaller than conventional magnetic particles and yet respond quickly to a magnetic field. This has an advantage that the smaller the particle, generally the higher the binding capacity. Accordingly, the invention allows the use of high capacity, small particles which

are still capable of obtaining a fast separation, as
compared with larger conventional particles. Particles
according to the invention are superior to paramagnetic
and superparamagnetic particles of the same size in terms
of velocity in a magnetic field. This is an enormous
advantage regarding isolation. In automatic systems it
becomes possible to increase the number of samples to be
analysed dramatically.

The magnetic material which forms part of the magnetic
particles is remanent in the sense that, upon exposure to
a magnetic field, the material must have residual
magnetisation in the absence of a magnetic field.
Accordingly, in the present specification remanence
encompasses both materials which have been previously
exposed to a magnetic field and therefore have residual
magnetisation and those materials which currently have no
residual magnetisation but will develop this feature
following exposure to the magnetic field. These
properties of magnetic materials according to the present
invention contrast those in the prior art such as
US6027945 or US5945525 in which the magnetic particles
are paramagnetic or superparamagnetic and are not
themselves magnetic in the absence of a magnetic field.

The magnetic material according to the present invention
advantageously comprises a ferrimagnetic material.
Whilst some texts define a ferrimagnetic material as one
which contains iron, according to the present
specification, a ferrimagnetic material is one which may
be a metal or a metal oxide and may or may not contain
iron. In one embodiment, the ferrimagnetic material

comprises a ferrimagnetic metal oxide which preferably comprises an iron oxide. Optionally all or a part of the ferrous iron of the metal or metal oxide may be substituted by a divalent transition metal selected from cadmium, chromium, cobalt, copper, magnesium, manganese, nickel, vanadium, and/or zinc. A particularly preferred ferrimagnetic metal oxide comprises ferrimagnetic magnetite.

In another embodiment of the present invention, the magnetic material is ferromagnetic, and preferably contains iron. The ferromagnetic material may be metal or metal oxide. Optionally, all or part of the iron of the metal or metal oxide may be substituted with another divalent transition metal as above.

The length or diameter of the magnetic particles is typically in the range 0.1 to 5,000µm, preferably in the range 0.1 to 1,000µm, more preferably in the range 0.1 to 500µm, most preferably in the range 0.1 to 100µm. It is found that smaller particles can be separated quickly in a magnetic field and will have high binding capacity. It is preferred that the magnetic particles are substantially spherical because particles of this shape disaggregate more easily.

The matrix material of the magnetic particles may comprise any material suitable to facilitate binding of the target substance. The composition of the matrix material will therefore depend to some extent on the nature of the target substance to be bound by the magnetic particles. The matrix material may provide a

coating or shell for the magnetic material and may bind or complex with the magnetic material or form a composite therewith.   In one arrangement the matrix material comprises a polymer which may be an organic polymer or an inorganic polymer such as a silica-based polymer.   Where the matrix material is inorganic, this may alternatively comprise salts or molecules.

It is advantageous for the surface of the magnetic particles to comprise functional groups which promote disaggregation of the magnetic particles in the presence of a liquid phase.   These functional groups may arise because of the nature of the matrix material used in the magnetic particles.   Alternatively, the matrix material may need to be treated in order to introduce those functional groups.   In one arrangement, the functional groups of the matrix material are hydrophilic for use with an aqueous liquid phase.   For example, where the aqueous liquid phase arises from a biological sample, a matrix material having a hydrophilic surface would be easier to disaggregate than a matrix material having a hydrophobic surface.   In the alternative, magnetic particles may be provided in which the functional groups of the matrix material are hydrophobic for use with an organic liquid phase, especially a non-polar liquid phase.   Where a non-polar liquid phase is used, a hydrophilic surface on the magnetic particles would make the particles more difficult to disaggregate.   It is also possible for the surface to have a combination of both hydrophilic and hydrophobic groups. Such a combination is preferred where solvent systems miscible with both water

and non-poplar solvents are used, such as THF, DIGLYMR
and DMSO.

The functional groups may also affect the binding
properties of the particles in relation to the target
substance. The capability of the magnetic particles to
bind the target substance may be conferred by the bulk
properties of the matrix material or by the matrix
material further comprising an affinant for binding the
target substance. Affinant chemistry and methodology is
discussed in further detail in "Immobilised Affinity
Ligand Techniques" by Hermanson et al (1992). The
surface properties and affinant properties of the
magnetic particles will be discusssed in further detail
below in relation to various different target substances.

In a further aspect the present invention provides a
process for the preparation of magnetic particles capable
of binding a target substance, which comprises providing
an unmagnetised magnetic material, and providing a matrix
material so as to form magnetic particles, wherein the
magnetic material is remanent upon exposure to a magnetic
field and the matrix material has a surface comprising
functional groups which promote disaggregation of the
particles in the presence of a liquid phase.

The matrix material may comprise a polymer which, as
discussed above, may be inorganic or organic. The
process may be performed in a number of ways. According
to one embodiment, the matrix material is provided
preformed and added to the magnetic material. According
to another embodiment, the polymer is preferably provided

by polymerisation of a monomer in the presence of an
unmagnetised magnetic material to form the magnetic
particles comprising the magnetic material and a
polymeric material. The monomer may comprise an organic
monomer or an inorganic monomer, such as a silica-based
monomer, depending on the desired polymer. Other
inorganic monomers include organometallic monomers,
sulfonitride monomers, phosphonitrilic monomers and
monomers to form carborane coordination polymers. This
polymerisation is not particularly limited but may
comprise a step-growth condensation (also called a
polyaddition reaction) and/or a radical reaction.

The polymerisation may take place in an emulsion in which
the unmagnetised magnetic material is present in
discontinuous phase thereof. According to this
embodiment, the step of polymerisation preferably takes
place in the discontinuous phase of the emulsion and the
monomer is typically also present in the discontinuous
phase of the emulsion, prior to polymerisation. The
present invention is not limited to this system since it
is also possible that some (or all) of the monomer may be
in the continuous phase. After a chemical reaction takes
place at the interface between the continuous and
discontinuous phase it is made possible for the monomer
to enter the emulsion droplets (discontinuous phase)
prior to the polymerisation. The emulsion may be water-
in-oil emulsion or an oil-in-water emulsion. Where the
emulsion is a water-in-oil emulsion, the monomer
generally comprises a water soluble organic and/or
inorganic monomer. Where the emulsion is an oil-in-water

emulsion, the monomer generally comprises a non-polar
organic and/or inorganic monomer.

As an alternative to an emulsion-based system, the step
of polymerisation may take place in solution followed by
a coating of the magnetic material.

The magnetic material may comprise particles, the length
or diameter of which is in the range 0.1μm to 5000μm,
preferably 0.1μm to 500μm and most preferably 0.1μm to
100μm. A particularly preferred length or diameter for
the magnetic material is in the range 100-300nm.

In use, the magnetic particles according to the invention
may be provided for separating a target substance from a
sample containing such a target substance. The target
substance may comprise a cell; a microorganism, which may
be cellular or acellular; a metal such as a pure metal or
compound comprising a minor or major part thereof; or an
organic compound such as an environmental contaminant, a
nucleic acid, or a protein.

One important target substance is a nucleic acid, which
may be DNA, RNA, or a modified form thereof. Where the
nucleic acid is DNA, this may be ds or ssDNA. Where the
nucleic acid is RNA, this may be rRNA, mRNA or total RNA.

A nucleic acid-containing sample typically comprises a
biological sample such as a cellular sample. The
biological sample may or may not need to be pretreated,
depending on its structure. For example, in the case of
plant or fungal cells or solid animal tissue,

pretreatment would be required as is known in the art.
Samples stored in the form of a solid phase such as a
paraffin section may also need pretreatment. Samples may
be from foodstuffs, environmental samples or clinical
samples and may contain prokaryotic or eukaryotic cells
or other moieties such as mycoplasmas, protoplasts or
viruses. Blood products are an important area for
nucleic acid isolation and the present invention is
particularly applicable to whole blood and other blood
products such as plasma, serum and buffycoat.

Where nucleic acid is to be purified, the matrix material
may comprise any material capable of binding nucleic
acid, such as certain organic polymeric materials or
silica-based materials. In one arrangement, the matrix
material bears acid groups on its surface as described in
GB0210766.2 filed on 10th May 2002 by the present
applicant company. The acid groups preferably comprise
an organic acid surface such as a carboxylic acid
surface.

Among those acid groups useable according to this aspect
of the present invention may be mentioned carboxy, sulpho
and aryloxy groups. For example, the carboxy or sulpho
groups may be linked to the solid phase by alkylene or
arylene groups so as to form carboxylic or sulphonic
acids. Aryloxy groups such as phenoxy groups may also be
so linked and may incorporate further aromatic or
aliphatic moieties. Carbon atoms in each type of organic
acid may be substituted with heteroatoms. The presence
of such heteroatoms and the optional presence of further
functional groups on the surface, including esters,

amines, alcohols, carboxylic acids, amides, halides,
aldehydes, ketones, imines, nitro compounds, thiols,
thioesters, nitriles, acid anhydrides and sulphonic
compounds may each contribute to the properties of the
solid phase, especially to the hydrophilicity of the
solid phase. The preferred solid phase is hydrophilic
because too hydrophobic a solid phase (for instance where
there is too a high a concentration of polystyrene) will
tend to give problems with nucleic acid binding.

Alternatively, the matrix material may comprise a silica-
based material for binding nucleic acid. Silica-based
magnetic particles may require the use of a chaotrope as
part of the isolation process to promote binding of the
nucleic acid to the particles.

The chaotrope generally comprises a chaotropic ion
provided at a concentration sufficiently high to cause
the nucleic acid to lose its secondary structure and, in
the case of double-stranded nucleic acids, to melt.
Chaotropes are thought to disrupt hydrogen-bonding in
water so as to make denatured nucleic acid more stable
than its undenatured counterpart. The chaotrope
typically comprises a guanidinium salt, urea, or an
iodide, chlorate, perchlorate or (iso)thiocyanate.
Preferred chaotropes include guanidinium thiocyanate, and
guanidinium hydrochloride.

The concentration of chaotrope typically present when
contacted with the sample is in the range 2M to 8M.

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In a further arrangement where the nucleic acid is the target substance, an affinant comprising an oligonucleotide may be used as a specific hybridisation probe for nucleic acid having a sequence complementary to the oligonucleotide sequence.

A step of separating the magnetic particles with the nucleic acid bound thereto from the liquid phase is generally required in order to remove contaminants in the liquid phase. Further washing steps may be applied to the solid phase at this point. Any conventional separation step for separating solid phase from liquid phase is applicable, including centrifugation and decanting of the liquid phase from the pelleted solid phase or using a column in which the solid phase is packed and the liquid phase passed through. Where the magnetic solid phase is used, this facilitates separation, which can be carried out in the presence of a magnetic field.

Depending on the form in which the isolated nucleic acid is required, a further elution step can be provided. In some cases it may be satisfactory for the nucleic acid to remain bound to the magnetic probe. This may be the case if further manipulations of the nucleic acid on a solid phase are required, such as an amplification step. Equally, the nucleic acid may be eluted from the solid phase by applying an elution solution, which may simply be water or a buffer.

According to further embodiments of the invention, the target substance may comprise a cell, protein, bacterium,

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virus, or environmental contaminant. The cells may be prokaryotic or eukaryotic cells. Eukaryotic cells include animal, plant and fungal cells. Prokaryotic cells include bacteria and blue green "algae". Other microorganisms include acellular microrganisms such as viruses and prions.

Suitable affinants may be selected which are known to bind each of these target substances. In one embodiment, the affinant is capable of binding a cell or a protein and preferably comprises an antibody, a binding protein, a fragment of an antibody or binding protein, or a ligand. The binding protein may comprise an avidin such as streptavidin or other biotin-binding affinant. According to this embodiment, the target substance is biotinylated. Alternatively, the avidin is bound to the target substance and the magnetic particles are biotinylated. In a further arrangement, the affinant comprises a ligand which comprises an oligonucleotide or a metal chelate specific for the target substance. The cell or protein may be microbial. The affinant may also be capable of binding a virus or a prion.

Where the target substance comprises cells, it is possible, for example, to introduce antibodies on the magnetic particles. The antibodies may be intact or present as an active fragment. Antibodies are typically introduced on the magnetic particles via covalent coupling of a ligand from the antibody to the surface of the magnetic particle, usually via the matrix material. Suitable ligands from the antibody include $-OH$, $-NH_2$ and $-SH$. Various coupling chemistries may be applied to

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couple the ligand of the antibody to the magnetic
particle.   For −OH it is possible for example to use
epoxy, divinyl sulfone, or cyanuric chloride.   For −SH,
it is possible to use maleimide, iodoacetyl, pyridyl
disulfide or epoxy activated matrices.   For −NH$_2$ coupling
it is possible to use epoxy, carboxylic acid/EDC,
azlactones, aldehydes/NaCNBH$_3$, cyanogen bromide, N−hydroxy
succinimides,   carbonyl diimidazoles,   organic sulfonyl
chlorides and others.

It is also possible to tailor the chemistry of the matrix
material so that it has affinity for the cell in
question.

As a further option, introduction of one of avidin or
biotin on the magnetic particles and introduction of the
other onto the cells will enable the particles
specifically to bind to the cells via an avidin−biotin
binding   interaction.   Typically   streptavidin is
introduced to the magnetic particles.   The cells may be
biotinylated for instance by using biotinylated NHS or by
allowing the cells to interact selectively with a reagent
which comprises biotin coupled to a moiety which reacts
specifically with the cells such as an antibody.

In the case where the target substance is a protein it is
possible to introduce protein binding proteins which
specifically target other proteins.   One example is to
introduce protein B on the magnetic particles to isolate
humane IgA (Faulmann et al 1991.   Equally, human IgA
could be introduced on the magnetic particles to isolate
protein B.

In another embodiment it is possible to isolate proteins
using the specific chemistry of the magnetic particles.
For example, oligonucleotides could be introduced on the
magnetic particles as affinants for specific amino acids
of the proteins.   Alternatively, it is possible to use
immobilised metal chelate affinity chromatography in
which chelates are introduced onto the magnetic particles
to isolate proteins via specific metal affinity domains
of the proteins.   One example of this is repeated
Histidine tags on proteins which will have an affinity
for immobilised nickel on the magnetic particles.

In a further embodiment, it is possible to use an
avidin/biotin binding pair in the same way as for
isolating cells.

Where the target substance comprises a microorganism such
as a virus, bacterium or other microorganism, one
strategy is to introduce antibodies or proteins on the
magnetic particles which have an affinity for the
proteins of the microorganism that are exposed on the
cell membrane or surface.   The methodology may be
analogous to that used in isolating other cells.
Alternatively, it is possible to introduce proteins that
have affinity for the microorganism proteins in the same
way as applied to isolating proteins as discussed above.

In a further embodiment, it is possible to use a
hydrophobic surface to obtain depletion of the bacteria
to that surface.

In a further embodiment, the target substance comprises a
metal and the affinant comprises a chelator for the
metal.

Where the target substance comprises a metal such as a
pure metal or metal compound which may be necessary to be
depleted from a sample for environmental reasons, it is
possible to introduce a metal chelator on the magnetic
particle. Examples include IDA or NTA for the specific
binding of metal of choice. Chelation chemistry is well
known to those skilled in this art and is discussed in
the book by Hermanson et al (1992).

Instead of using an affinant to bind microorganisms, the
matrix material may comprise a hydrophobic functional
group capable of binding the microorganisms. Hydrophobic
functional groups may also be used on the matrix material
in order to bind hydrophobic target substances such as
environmental contaminants. For example, PCBs have a
hydrophobic structure which is capable of being bound by
a hydrophobic surface on a matrix material. The
hydrophobic surface may, for example, be obtained by
using aromatic groups.

The magnetic particles according to the present invention
may be used in a positive selection or a negative
selection of the target substance. In a positive
selection, the target substance is required for further
use or further isolation and possibly purification. In
positive selection it is preferred to avoid non-specific
isolation of contaminating material. Easy disaggregation
of the magnetic particles is extremely important in

positive isolation to ensure good mixing and facilitate efficient washing of the particles with the target substance bound thereto. Isolation of nucleic acid is just one example of positive selection where the target substance is isolated from the sample.

In a negative selection, the target substance is depleted from the sample. The purpose of this is generally to clean the sample for future manipulation or use of the sample. Removal of contaminants such as environmental contaminants is one example of a negative selection. Another example of negative selection is where the target substance is T-cells and the sample is a blood sample.

The magnetic particles may be used in a cell sorting apparatus for positive selection or negative selection.

In a further aspect, the present invention provides a process for separating a target substance from a target substance containing sample, which comprises:

(a) providing target substance binding magnetic particles which comprise a magnetic material and a matrix material, wherein the magnetic material is remnant upon exposure to a magnetic field;

(b) providing a liquid phase comprising the target substance-containing sample;

(c) dispersing the sample with the magnetic particles so as to bind the target substance thereto; and

(d) isolating the particles from the sample by applying a magnetic field thereto and separating the particles from the liquid phase.

The step of dispersing the sample with the magnetic particles preferably comprises subjecting the magnetic particles to disruption to disaggregate the particles. The disruption may comprise mechanical, acoustic or UV disruption. Mechanical disruption includes pipetting, stirring, vortexing and/or shaking so as to disaggregate the particles. Acoustic disruption includes ultra sonication and UV disruption. It is important that the sample is dispersed as fully as possible with the magnetic particles so as to maximise binding of the target substance thereto.

The process is useful for separating a target substance as defined above and may be used in a positive selection or a negative selection. Isolation of nucleic acid is a particularly important aspect of the invention, especially isolation of unfractionated nucleic acid such as total nucleic acid from a biological sample.

The process of the invention may include further steps. For example, where the isolated target substance is to be further purified or used in further manipulation, one or more washing steps may be incorporated into the process following binding of the target substance to the magnetic particles. In some cases the target substance may be used in a state bound to the magnetic particles. In other cases, there is a need to elute the target substance from the magnetic particles, for example, by applying an elution solution.

In a further aspect, the present invention provides a kit for separating a target substance from a sample

containing such a target substance. The kit comprises magnetic particles as defined herein typically dispersed in a buffered aqueous solution and optionally including a component to inhibit microbial growth such as an azide. Sodium azide at 0.02% is a typical additive in such a buffered aqueous solution. The kit may typically further comprise one or more binding solutions, one or more washing solutions and one or more elution solutions each of which is generally aqueous. The elution solution may be aqueous or non-aqueous, depending on the target substance. Where samples require pretreatment, for example where biological samples incorporate material to be lysed, the kit will additionally include one or more lysis solutions. Where nucleic acid is the target substance, the kit may appear in a standard format comprising a nucleic acid binding magnetic particle, together with one or more of the solutions discussed above. Where the nucleic acid binding magnetic particle is a silica magnetic particle, the kit may also include a chaotrope.

Detailed Description of the Invention
The present invention is now described in more detail, by way of example only, with reference to the following Examples.

Examples
All Examples are performed in the absence of an applied magnetic field.

Example 1

In this example, an aqueous dispersion of ferrimagnetic magnetite particles in sodium silicate solution (water glass) is mixed with an oil phase to form a water-in-oil emulsion with magnetite in the aqueous phase. Condensation polymerisation is performed in the presence of acid to produce the magnetic particles with an inorganic polymer.

Ferrimagnetic magnetite particles (size 200-300 nm) 20g were dispersed in 40g waterglass (NMD) using an ultraturax mixing device. After mixing for 1 min at 16000 rpm, the speed was reduced to 13000 rpm and 300 ml of an oilphase (for instance toluene or isopar) containing 3% of an emulsifier (for instance span 80, span 65) was added. The speed was increased to 1700 rpm for 1 min and the resulting water in oil emulsion (magnetite dispersed in the water phase) was stirred in a reactor for 10 min at 20°C before 2M HNO_3 (30 ml) was added. After stirring for 1h and addition of methanol (30 ml), the suspension was stirred at 50°C for 16h. The magnetic particles were washed with methanol (3x150 ml), water (1x150ml) and finally methanol (2x150 ml) using a centrifuge or a magnetic device. The particles were dried under vacuum. Particle size 0,3μm – 1,5μm. Relative susceptibility: 35 x 10^{-3} cgs.

Example 2

In this example ferrimagnetic magnetite particles are dispersed in an organic monomer (EGDMA) and an oil in water emulsion is formed by mixing the particle suspension with an aqueous phase. The monomers are

polymerised to produce the organic polymer magnetic
particles.

Ferrimagnetic magnetite particles (size 200 - 300 nm)
6,6g were dispersed in 20g EGDMA. AIBN (0,45g) was added
to the dispersion and the organic phase containing
magnetite was emulsified in water (150 ml) containing
0,5% polyvinylalcohol (Evanol) by use of an ultraturax
(13000 rpm, 2 min). The resulting emulsion was stirred
in an reactor for 20h at 65°C and the magnetic polymer
beads were washed with methanol (5x150 ml) and dried at
80°C for 6h. Particle size 0,7μm - 6μm. Relative
susceptibility: 15×10^{-3} cgs.

Example 3.

In this Example ferrimagnetic magnetic particles are
dispersed in an organic solvent with a monomer, which is
then polymerised to form the particles.

Magnetite (1g) is dispersed in an organic solvent such as
THF, hexane or toluene (10 ml), where after an epoxiresin
like bisphenol-A (10 ml) is added. Stirring is continued
at 70°C for 16 h and the magnetic particle are then
washed 5 times with THF (25 ml each wash) by using a
centrifuge. Finally the particles are dried in vacuum at
50°C. The particles have approximately 0.25 mmol/g
epoxigroups.

Example 4.

In this Example ferrimagnetic magnetic particles are dispersed in an organic solvent with a prepolymerised polymer to form the particles.

Dry ferrimagnetic magnetite particles (size 200–300 nm) 1 g were dispersed in 10 ml of 0.5% poly(ethylene) imine (Aldrich, Mw 35 000) in 0.1 M Na-carbonate pH 9.5. The suspension was allowed to incubate at ambient temperature for 3 h, where after the particles were washed with 4 x 20 ml water.

Introduced polymers were confirmed by surface charge measurements (Malvern Zetaziser). The surface of the magnetic particle had a positive shift in isoelectric point of 1 magnitude.